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Microarray analysis of cultured chondrocytes and adipose-derived adult stem cells

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Purpose: To address both basic and applied research problems related to autologous chondrocyte implantation, we developed a three-phase culture process to tissue engineer neocartilage from chondrocytes or adipose-derived adult stem (ADAS) cells. However the quality of in vitro generated neocartilage, in particular from ADAS cells, is currently limited by an insufficient understanding of the regulatory roles of specific molecular signals on tissue development. To investigate the initial period of differentiation, gene expression was compared between cultured chondrocytes and ADAS cells by microarray analysis.
Methods and Materials: Chondrocytes and ADAS cells were extracted from biopsies of articular cartilage and infrapatellar fat tissue taken from the stifle joint of sheep. RNA extracted from cultured cells was amplified, labelled and hybridised to human oligonucleotide microarrays representing 20,000 genes. Data acquisition and management was conducted according to MIAME protocols. The expression of differentially regulated genes will be confirmed by quantitative RT-PCR.
Results: Microarray data analysis identified 88 genes up regulated more than two-fold with respect to each cell type. Fifty four genes were specific to chondrocytes and 34 genes were specific to ADAS cells. Chondrocytes showed higher expression of cell growth and cell cycle genes, and type I collagen. In contrast, ADAS cells showed higher expression of WNT inhibitory factor, fibronectin, collagen type 11 and type 12, and 10 cDNAs of unknown function.
Conclusions: This preliminary data highlights differences in gene expression between chondrocytes and ADAS cells, which suggest that specific culture regimes to differentiate ADAS cells are required for successful neocartilage production.

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3D pellet mass co-culturing system with human embryonic stem cells and human Chondrocytes induces Chondrogenic differentiation

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Purpose: Human embryonic stem (hES) cells are pluripotent cells and have the capability to differentiate to various cell types. The hES cells are suggested as the ultimate source for cell based therapies and may represent an alternative cell source for the treatment of cartilage defects. Chondrogenic differentiation of hES cells are the purpose of this study.
Methods and Materials: Here we show that in vitro differentiation of hES cells (SA167 and AS034.1, Cellartis AB, Gothenburg, Sweden) toward the chondrogenic lineage can be achieved through co-culturing of hES cells and chondrocytes in 3D pellet mass culturing system. The result verifies that these differentiated hES cells have the potential to differentiate towards chondrogenic cell types and share some qualities that are characteristic for chondrocytes. In vitro characterization of the co-culture differentiated hES cells was preformed based on their morphology, ability for osteogenic differentiation, ability to form clones in a chondrogenic selective culture condition, ability to form a pellet and produce matrix in a 3D culturing system.
Results: The histological and immunohistochemical analysis of the resulted pellets from the co-culture differentiated hES cells revealed, collagen type 1, chondroitin 4-sulfate and chondroitin 6-sulfate expression. Von Kossa staining indicated mineral deposition in osteogenic assay. Furthermore, phase-contrast microscopy and transmission electron microscopy confirmed matrix production in 3D cultures. Appearance of clones in agarose cultures is yet another proof for chondrogenic differentiation.
Conclusions: So far all results point out that directed co-culture could have a strong chondrogenic effect on undifferentiated hES cells. This directed co-culture model can be useful for studying early chondrogenesis.

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Phenotypic analysis of changes in cell surface markers and gene expression of human mesenchymal stem cells and chondrocytes during monolayer expansion

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Purpose: Both chondrocytes and mensenchymal stem cells (MSCs) are the most used cell sources for cartilage tissue engineering. Monolayer expansion to obtain sufficient cells leads to rapid dedifferentiation of chondrocytes and, concomitantly, reduced ability of MSCs to differentiate into chondrocytes, limiting their application in cartilage repair. The aim of this study was to investigate the influence of the monolayer expansion on the phenotype and the gene expression profile of both cell types, and to find the appropriate compromise between monolayer expansion and the retaining structure of chondrogenic characteristics.
Methods and Materials: Human chondrocytes, isolated enzymatically from femoral head, and human MSCs, derived from bone marrow, were maintained in monolayer culture up to passage 5. The expressions of cell surface markers (CD34, CD45, CD73, CD90, CD105, CD166) and several chondrogenic-related genes for each passage of those cells were then analyzed using flow cytometry and quantitative RTPCR.
Results: Flow cytometry analyses showed that, during the monolayer expansion, some qualitative and quantitative regulation occur for the expression of cell surface markers. Chondrocytic expression pattern is similar to those for MSCs. A rapid increase in mRNA expression of type I collagen and aggrecan occurs whereas a significant decrease of type II collagen and sox 9 was observed in chondrocytes through the successive passages. The expansion did not induced obvious change in MSCs gene expression.
Conclusions: Our results suggest that passage 2 might be the up-limit for chondrocytes to achieve their redifferentiation in 3D scaffold. Nevertheless, MSCs could be expanded in monolayer until passage 5 without loss of their undifferentiated phenotypes.

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Replication of development recreates the fetal cascade from stem cell to articular cartilage in adults.

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Purpose: Articular cartilage is a remnant of the fetal cartilagenous skeleton and it is necessary to regenerate it by replication of the original fetal developmental cascade. The author has accomplished this in adults with the use of a unique action of purified growth hormone.
Methods and Materials: Trochlear surfaces of mature New Zealand rabbits were surgically debrided to bleeding bone. Purified growth hormone was injected into one knee joint and saline of the same pH was injected into the other knee. Sacrifice was carried out at intervals and transverse sections of the trochlear surface were removed and processed for study with routine, phase contrast, and Hoffman Modulation Contrast microscopy.
Results: In the hormone injected knees the cascade of fetal development started with the appearance of cartilage canals composed of fenestrated capillaries which produced stem cells. The stem cells passed through the fenestrations into matrix and were signaled to form chondrocytes. Vertical microfibrils and arcades also formed. The new surface was firmly bonded to the host bone surface from which it originated. Inflammation was absent in this developmental replication. The saline injected knees healed with fibrocartilage and scar tissue.
Conclusions: The author regenerated articular cartilage by duplicating the fetal developmental cascade in adults. He accomplished this by utilizing a unique action of growth hormone. Inflammation was absent in this developmental cascade which represents pre-natal healing. Problems associated with other attempts to regrow articular cartilage were not observed in the hormone treated knees. The saline injected knees healed with fibosis and fibrocartilage which represent post-natal healing.